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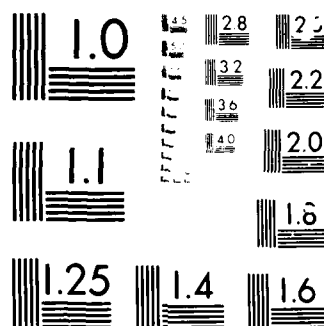
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The underlying premise of this research is that the neuron itself is the functional unit in the brain for positive reinforcement. Our early studies demonstrated for the first time that the firing rate of a brain cell could be increased by local applications of reinforcing transmitters or drugs. Our current work has two aims: 1) to examine the detailed anatomical and pharmacological properties of such cellular operant conditioning, and 2) to compare these properties with those of behavioral operant conditioning in order to determine important similarities and differences. We have studied cellular operant conditioning in whole-brain and brain-slice experiments. In whole brain, we have attempted to identify those cells most susceptible to reinforcement using electrical stimulation of rewarding brain sites as reinforcement. In brain slice experiments, we have found that 1) the reinforcing action of dopamine is likely mediated at D2 dopamine receptors, and 2) cellular operant conditioning is possible using locally applied electrical stimulation as reinforcement. At the behavioral level we have continued our pharmacological characterization of reinforcement receptors in self-stimulation of hippocampus and nucleus accumbens (primary sites of the brain slice experiments), and characterization of reinforcement receptors in place preference studies (an alternative method to self-stimulation for measuring reward).					
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NEURONAL MECHANISMS OF INTELLIGENCE

DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF CALIFORNIA, IRVINE
IRVINE, CA 92717

L. Stein & J.D. Belluzzi

Controlling Office: USAF Office of Scientific Research/NL
Bolling Air Force Base, DC 20332

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Good progress has been achieved this year in our continuing studies of the cellular basis of reinforced (intelligent) behavior. The underlying premise of this research is that the neuron itself is the functional unit in the brain for positive reinforcement. Our early studies demonstrated for the first time that the firing rate of a brain cell could be increased by local applications of reinforcing transmitters or drugs. Our current work has two aims: 1) to examine the detailed anatomical and pharmacological properties of such cellular operant conditioning, and 2) to compare these properties with those of behavioral operant conditioning in order to determine important similarities and differences. The work is organized into the following projects:

At the cellular level:

- a) in whole brain experiments, identification of those cells most susceptible to reinforcement using electrical stimulation of rewarding brain sites as reinforcement,
- b) in brain slice experiments, pharmacological characterization of reinforcement receptors,
- c) in brain slice experiments, demonstration of cellular operant conditioning in brain slices using locally applied electrical stimulation as reinforcement,
- d) in brain slice experiments, examination of the role of calcium transport in cellular operant conditioning.

At the behavioral level:

- a) pharmacological characterization of reinforcement receptors in self-stimulation of hippocampus and nucleus accumbens (primary sites of the brain slice experiments),
- b) characterization of reinforcement receptors in place preference studies (an alternative method to self-stimulation for measuring reward).

A brief description of current results and future experiments is given below.

1. Operant conditioning of individual neurons.

a. Reinforcement receptors in single-unit operant conditioning.

We have continued our analysis of operant conditioning of individual CA1 cellular activity in slices of hippocampus, using local applications of dopamine as reinforcement. During the past year we have examined the pharmacological specificity of such cellular operant conditioning and have tentatively identified the "reinforcement" receptors that may be involved. Using our standard operant conditioning paradigm with dopamine applications as reinforcement, we have studied the effectiveness of various dopamine receptor antagonists. Chlorpromazine, a mixed dopamine D₁ and D₂ receptor antagonist, completely blocked dopamine's effectiveness in producing operant conditioning (Fig. 1).

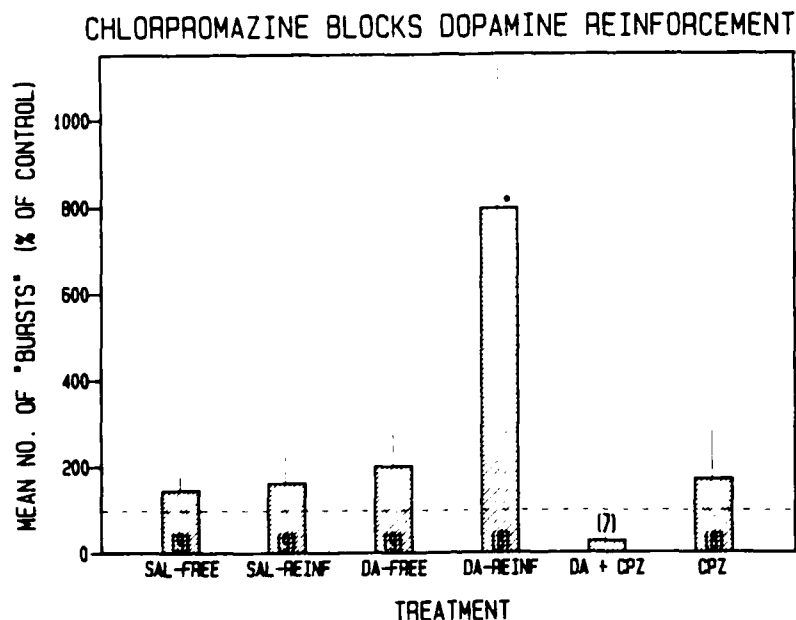


Figure 1. Chlorpromazine blocks operant conditioning of individual CA1 cellular activity in slices of hippocampus, using local applications of dopamine as reinforcement. A single-barrelled glass micropipette for simultaneous recording and pressure injection was filled with dopamine (1 mM in 165 mM saline) and aimed at spontaneously active pyramidal cells in the CA1 layer of hippocampal slices. The neuronal response for reinforcement was a burst of relatively fast activity. To be eligible for reinforcement, such "bursts" had to contain a minimum number of spikes; this minimum number was individually established for each neuron studied so that, prior to operant conditioning, reinforceable "bursts" occurred at a rate of approximately 5 per minute. For reinforcement, the pressure injector was activated for 10-80 ms immediately after each "burst" to deliver a 10 μ -diameter droplet of drug to the cell. Neurons reinforced with dopamine (DA-REINF) exhibited significantly more "bursts" than controls reinforced with saline (SAL-REINF). When chlorpromazine (1 mM) was added to the dopamine solution (DA + CPZ), the reinforcing action of dopamine was abolished and the rate of "bursts" was suppressed below the saline control. On the other hand, neurons that received chlorpromazine alone (CPZ) exhibited the same number of "bursts" as those that had received saline. SAL-FREE = noncontingent saline injections.

A closer analysis of receptor specificity was performed in an experiment similar to Fig. 1 above, but using antagonists with greater receptor selectivity

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than chlorpromazine. Sulpiride, a D_2 dopamine receptor antagonist, and SCH 23390, a D_1 dopamine receptor antagonist, were used separately and in combination with dopamine in cellular operant conditioning experiments. The results (Fig. 2) show that D_2 receptor blockade by sulpiride reduced conditioning (DA + SUL), whereas D_1 receptor antagonism (DA + SCH) had no effect.

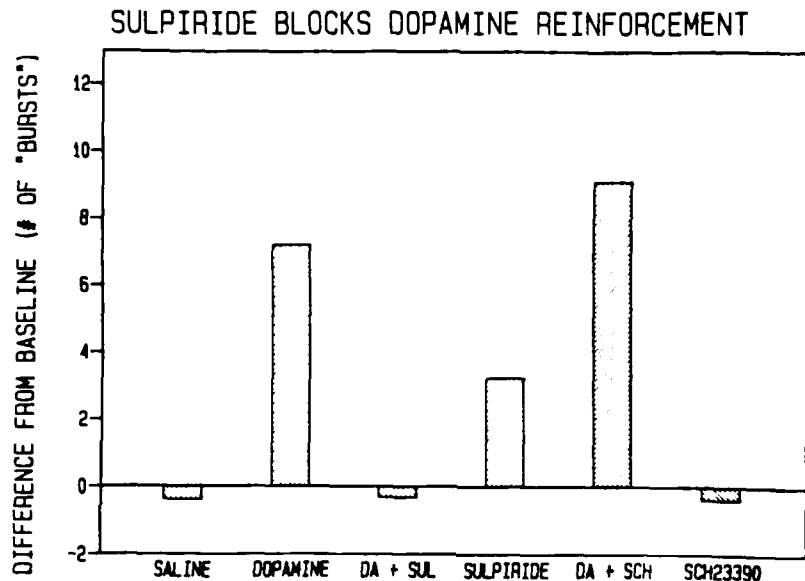


Figure 2. Sulpiride, but not SCH 23390, blocks operant conditioning of individual CA1 cellular activity in slices of hippocampus, using applications of dopamine as reinforcement. Methods are the same as in Fig. 1. DA + SUL = 1 mM dopamine plus 10 mM sulpiride; SULPIRIDE = 10 mM sulpiride; DA + SCH = 1 mM dopamine plus 1 mM SCH 23390; SCH23390 = 1 mM SCH 23390.

These results replicate our earlier findings that dopamine is a highly effective reinforcing agent for single-unit operant conditioning, and they strongly suggest that the reinforcing action of dopamine is mediated at D_2 dopamine receptors. We intend to use similar procedures to analyze cellular conditioning of other cell types.

In a related experiment, we attempted to increase the sensitivity of endogenous dopamine receptors by chronically treating rats with haloperidol and testing for conditioning eight days after the end of haloperidol treatment when dopamine receptors are supersensitive. We reasoned that dopamine reinforcement might be more effective with supersensitive receptor. However,

when brain slices were made from these rats no differences in conditioning were found when compared with normal controls.

b. Single-unit operant conditioning with electrical stimulation as reinforcement in brain slice.

Nucleus accumbens cells are the targets of major dopamine projections from rewarding brain areas. We have prepared brain slices containing both nucleus accumbens cells and dopamine projections and have attempted to condition the activity of nucleus accumbens cells using electrical stimulation of the dopamine projections as reinforcement. We found that high-rate activity of nucleus accumbens neurons could be increased with reinforcing electrical stimulation in the dopamine projections, whereas noncontingent stimulation of similar intensity and duration had no effect on neuronal activity (Fig. 3).

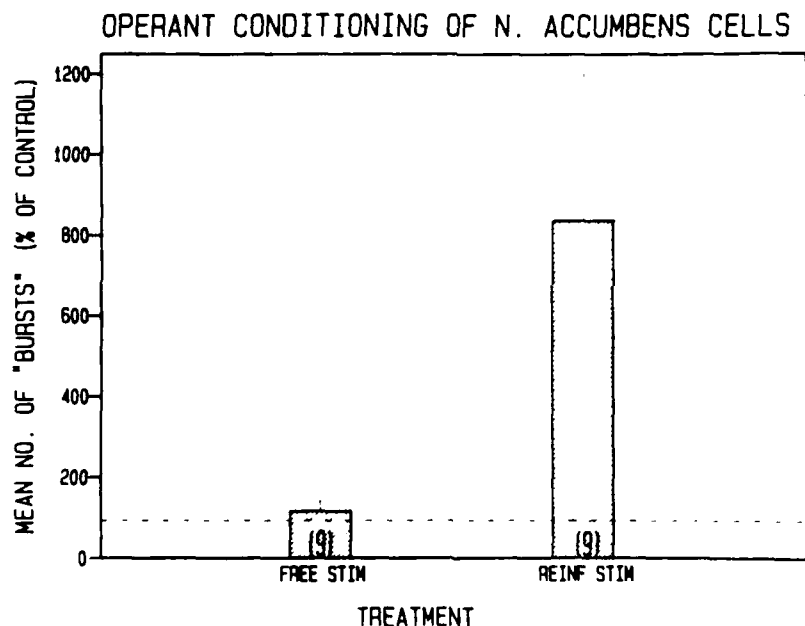


Figure 3. High-rate activity of neurons in the vicinity of nucleus accumbens in brain slice was reinforced with electrical stimulation in adjacent areas presumed to contain dopamine fibers projecting to nucleus accumbens cells. Stimulation (50-100 μ A, 100 Hz, 100 ms) contingent on high-rate firing caused increased occurrence of high-rate activity (REINF STIM), whereas noncontingent stimulation of similar intensity and duration had no effect on neuronal activity (FREE STIM).

These results support the idea that synaptic release of dopamine may be involved in the reinforcement process. Further, this method provides a

promising approach for the analysis of other target cells of dopamine projections, and we intend to continue this line of research.

c. Single-unit operant conditioning in whole brain using rewarding medial forebrain bundle stimulation as reinforcement.

We are studying whole brain preparations in order to identify target cells of the endogenous reward system for further analysis both in whole brain and in brain slice. Rats had electrodes chronically implanted into the medial forebrain bundle (MFB) and were tested for self-stimulation. Positive responders were anesthetized with urethane (1.2 g/kg, I.P.), an extracellular recording electrode was lowered into cortex, dorsal to nucleus accumbens (a major target for the dopamine fibers in the MFB), and individual neurons were conditioned using MFB stimulation as reinforcement. To date we have found conditionable neurons in nucleus accumbens and medial frontal cortex (Fig. 4).

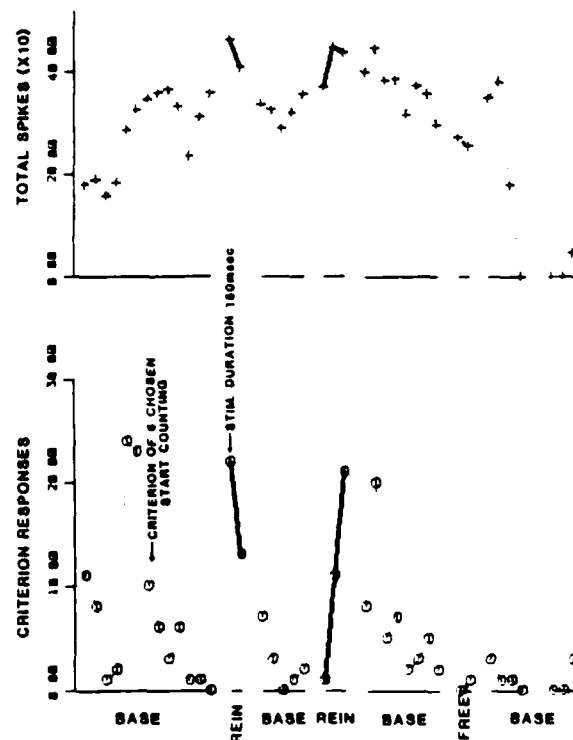


Figure 4. Operant conditioning of cortical cellular activity in an anesthetized rat using stimulation of medial forebrain bundle as reinforcement. Cellular criterion responses were defined as 6 consecutive spikes with 15 ms maximum interspike interval. Reinforcement consisted of electrical stimulation of the MFB (150 msec train of 0.2 msec pulses at 100 Hz, 400 μ A) and resulted in a large increase in criterion responses (REIN) over baseline rate. Noncontingent stimulation at the same density, however, clearly decreased response rate (FREE).

Such conditioning has been observed in 4 of 12 cortical neurons. Other brain areas, such as nucleus accumbens, also are under study. This information is being used to develop brain slices containing similar neuronal populations for operant conditioning studies using direct chemical and electrical reinforcements.

d. Calcium mechanisms in single-unit operant conditioning.

Experiments are in progress to examine the regulation of brain calcium channels. In brain slice experiments, an uncharacterized factor isolated from rat brain produced membrane depolarization in CA1 hippocampal cells which (a) was dependent on the presence of calcium in the medium, (b) was reversed by application of cobalt ions, which block calcium channels, and (c) was not affected by tetrodotoxin pretreatments, which block sodium channels. These results suggest that brain calcium channels in hippocampus are pharmacologically regulated, and that such regulation of calcium channel activation may be subject to physiological regulation by factors such as learning and reinforcement. We are currently exploring a new method developed by Dan Johnston in which hippocampal slices can be "unzippered" to expose CA1 cell bodies for more detailed study.

Related experiments have just begun to determine the structure of the binding site of the drug diltiazem (a calcium entry blocker) on brain calcium channels. Radioactive diltiazem has been incubated with rat brain membranes, which are then exposed to ultraviolet light to produce photoactivation of the drug and a resulting covalent drug-membrane interaction. Initial results show that photoactivation can be achieved, and this should eventually allow the determination of the molecular weight of the diltiazem binding site on the brain calcium channel.

2. Brain self-stimulation---receptor specificity.

a. Pharmacology of hippocampal self-stimulation.

The success of operant conditioning of hippocampal cells with dopamine reinforcement led us to reexamine hippocampal self-stimulation for anatomical and pharmacological specificity. Rats with bipolar electrodes aimed at various areas of hippocampus were trained in a nose-poke self-stimulation task. Target sites for electrode implantation were ventral hippocampus (VH), thought to be more highly innervated by dopamine projections, dentate gyrus (DG), and CA1 and CA3 fields of the dorsal hippocampus. Rats were trained in self-stimulation to a stable response rate, and drugs were then administered (see Table 1), one per week, to determine catecholamine or endorphin involvement in self-stimulation. Amphetamine caused a statistically significant increase in response rate at three of the four electrode sites tested (see Table 1). Generally, all drugs caused changes in response rate in the direction expected from other self-stimulation work, but the changes tended to be small and variable, possibly due to low baseline self-stimulation rates. These results suggest that hippocampal self-stimulation is predominantly dopaminergic, as is the case in our cellular operant conditioning experiments in hippocampal slices.

Table 1. Effects of various drugs on hippocampal self-stimulation.

Drug	Dose (mg/kg)	Change in self-stimulation rate (mean \pm SEM)			
		CA1	CA3	VH	DG
Amphetamine	1.0	857 \pm 243**	383 \pm 96**	424 \pm 215	1079 \pm 279 ⁺
Morphine	1.0	97 \pm 58	12 \pm 44	38 \pm 41	2 \pm 70
Naloxone	2.0	-9 \pm 14	-88 \pm 33*	-104 \pm 71	-105 \pm 35
Haloperidol	0.05	-82 \pm 46	-131 \pm 94	-138 \pm 104	19 \pm 89

* $p < 0.05$; ** $p < 0.02$; + $p < 0.01$

b. Naloxone suppresses self-stimulation by central blockade of reinforcement.

Opiate receptor antagonists, such as naloxone and naltrexone, suppress self-stimulation of enkephalin-rich brain regions following peripheral administration. According to the endorphin theory of reinforcement, these antagonists suppress self-stimulation by blocking central opiate receptors, thereby preventing the reinforcing action of stimulation-released endogenous opioids (Belluzzi & Stein, 1977). However, since self-stimulation can be suppressed by effects unrelated to reinforcement, others have suggested that these drugs may produce nonspecific performance deficits. Therefore, we tested whether opiate antagonists interfere with reinforcement or performance in self-stimulation. Since one would expect reinforcing effects of brain stimulation to be centrally mediated, the effects of opiate antagonists that do not cross the blood-brain barrier were studied. Rats were trained to lever-press for nucleus accumbens brain stimulation in 1-hr daily sessions. When stable baseline rates were obtained, naloxone was compared to its quaternary derivative, naloxone methobromide, for potency to suppress self-stimulation. Naloxone methobromide does not cross the blood-brain barrier and therefore blocks only peripheral opiate receptors. Naloxone caused significant suppression of self-stimulation at all doses tested (Fig. 5), whereas even the highest dose of naloxone methobromide examined (20 mg/kg s.c.) had no effect. In similar experiments, naltrexone (20 mg/kg s.c.) produced a strong suppression whereas the same dose of naltrexone methobromide had no effect. These findings suggest that opiate antagonists suppress self-stimulation by blocking opiate receptors in the brain.

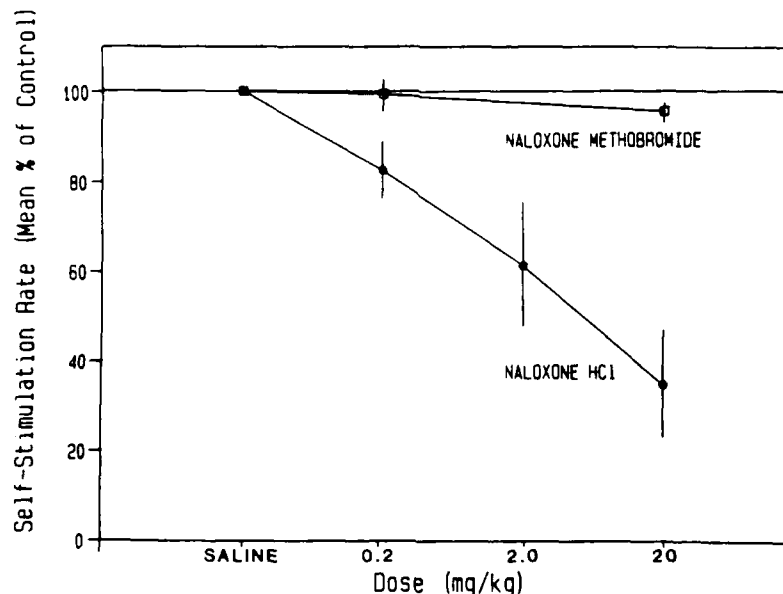


Figure 5. Effects of naloxone HCl on self-stimulation of nucleus accumbens. Naloxone was administered immediately before the daily 1 hr self-stimulation session and means were computed for response rate during the last 45 min. Each point represents the mean \pm S.E.M. for 8-11 animals. Naloxone HCl significantly suppressed self-stimulation at all doses tested. Naloxone methobromide had no effect at either dose tested.

c. Naloxone suppression of self-stimulation is independent of response difficulty.

To determine if opiate antagonists suppress self-stimulation by interfering with the ability of the animals to respond, the effects of naloxone were compared on lever-pressing and nose-poking for nucleus accumbens self-stimulation. Nose-poking is a simple response requiring little motor output, whereas lever-pressing is more difficult. If opiate antagonists act by interfering with motor capacity, then nose-poking should be relatively unaffected by naloxone. On the otherhand, if these drugs act by blocking reinforcement, then nose-poking and lever-pressing should be equally suppressed. Naloxone (0.2, 2.0, and 20 mg/kg s.c.) suppressed nose-poking and lever-pressing for self-stimulation equally (Fig. 6). These results suggest that opiate antagonists suppress self-stimulation by interfering with central

reinforcement rather than motor output, and support the role of endogenous opioids in brain stimulation reward.

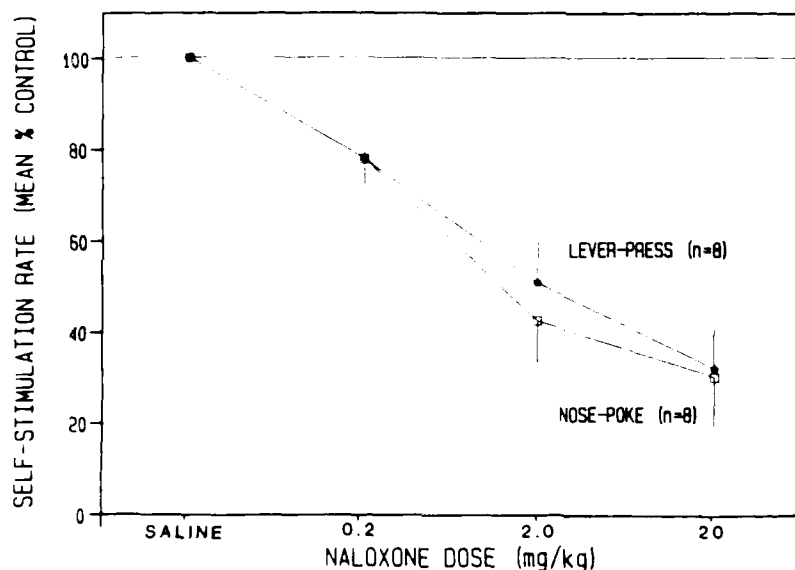


Figure 6. Effects of naloxone HCl on nose-poking and level-pressing for self-stimulation of nucleus accumbens. Naloxone was administered immediately before the daily 1 hr self-stimulation session and means were computed for response rate during the last 45 min. Each point represents the mean \pm S.E.M. for 8 animals. Naloxone HCl significantly suppressed self-stimulation equally for both tasks at all doses tested.

3. Conditioned place preference experiments.

a. Naloxone blockade of amphetamine reward in place preference conditioning.

The conditioned place preference paradigm was used to study possible interactions between endogenous opioids and catecholamines in reinforcement. Evidence suggests that these neurotransmitters are involved in reinforcement, and may serve as reinforcement transmitters in the brain. In addition, studies suggest that the endogenous opioids and catecholamines may interact in reinforcement. In the present studies amphetamine, which potentiates the release of catecholamines, and naloxone, a potent and selective opiate receptor antagonist, were examined alone and in combination in conditioned place preference. Two identical shuttle boxes were used in which

two compartments are distinguished by color, odor, and texture. Initial compartment preferences were determined by measuring the time spent in each compartment for four baseline days. Rats then were conditioned by pairing one compartment with drug and the other with saline over eight 30 minute sessions. The reinforcing or aversive properties of a drug were determined in post-conditioning tests by measuring the change in compartment preference from baseline. Reinforcing effects were assessed by pairing drug injections with the initially non-preferred compartment, while aversive effects were assessed by pairing drug with the initially preferred compartment. As observed in previous studies, amphetamine (1.0 mg/kg, s.c.) shifted place preference to the compartment associated with drug, demonstrating amphetamine's reinforcing properties (Fig. 7). Naloxone (2.0, 0.2, & 0.02mg/kg, s.c.) administered with

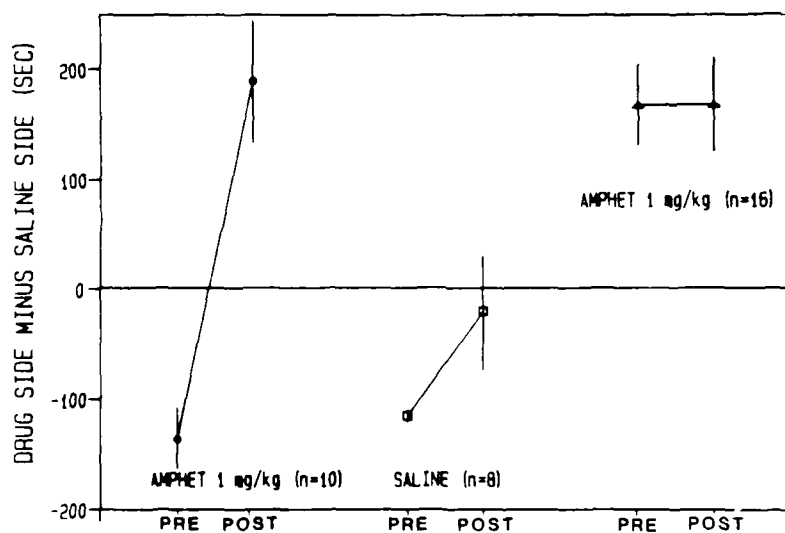


Figure 7. Effects of amphetamine (1.0 mg/kg, s.c.) in conditioned place preference. Amphetamine paired with the initially non-preferred compartment (left graph) caused animals to shift their preference to this compartment. Saline paired with the initially non-preferred compartment (middle graph) caused the animals to prefer neither compartment. Amphetamine paired with the initially preferred compartment (right graph) did not produce any shift in preference.

amphetamine blocked the preference for the compartment paired with amphetamine (Fig. 8). These data suggest that amphetamine is indeed reinforcing, and that opiate receptor blockade can prevent the reinforcing

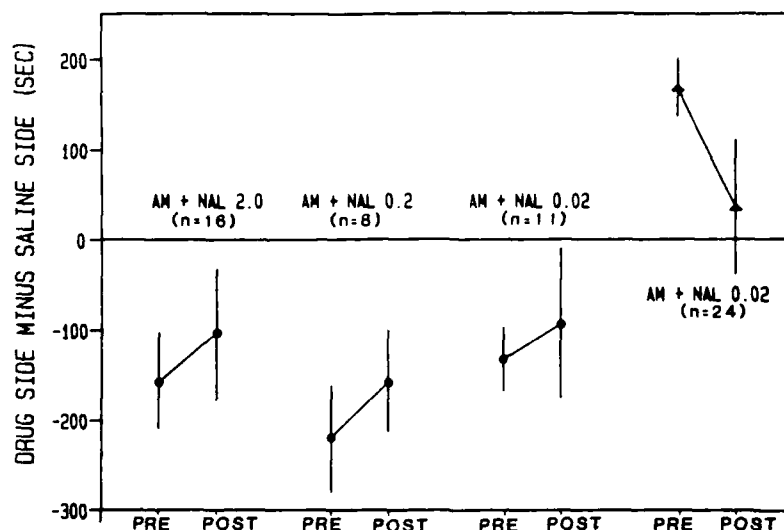


Figure 8. Naloxone blocks amphetamine-induced conditioned place preference. When a mixture of naloxone (0.02, 0.2 or 2 mg/kg) and amphetamine (1.0 mg/kg, s.c.) was paired with the initially non-preferred compartment no shift in preference was observed (compare Fig. 7). When amphetamine and 0.02 mg/kg of naloxone were paired with the initially preferred compartment (right graph) no significant shift in preference was observed.

properties of amphetamine. Furthermore, these results suggest that endogenous opioids may be important in the reinforcing effects of amphetamine, and support the possibility of interactions between endogenous opioids and catecholamines in reinforcement.

b. Sulpiride facilitation of amphetamine conditioned place preference.

In a related experiment, we found that sulpiride significantly potentiated amphetamine conditioned place preference. Sulpiride has high affinity for presynaptic dopamine D_2 receptors. Amphetamine is believed to be reinforcing, at least in part, through its effect to increase synaptic dopamine levels. Therefore, the results suggest that blockade of presynaptic feedback inhibition of dopamine release facilitates amphetamine's effects. We are conducting further studies in conditioned place preference and brain self-stimulation using specific dopamine D_1 and D_2 agonists and antagonists to attempt to identify the reinforcing dopamine receptor subtype.

4. Project Personnel.

Late in 1984 we hired Ms. Eve Chan to help perform neurophysiological experiments. She has made a strong contribution to the project. Mr. Joel Black continues his work on project research. In fall 1985 Dr. Dave Gilbert joined our group and is working on behavioral experiments related to the project. Ms. Karen Stevens is working part time on project research. Dr. Gilbert, and graduate students Black and Stevens all have separate support. Dr. Keith Trujillo received his Ph.D. degree in fall 1985 and has taken a postdoctoral position in the laboratory of Dr. Huda Akil at the University of Michigan.

5. Project Publications.

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- Trujillo, K.A., Tabrizi, P.R., Belluzzi, J.D. & Stein, L. Naloxone blockade of amphetamine reward in place preference conditioning. *Soc. Neurosci. Abst.*, 1985, 11, 1173.
- Stein, L. & Belluzzi, J.D. Operant conditioning of individual neurons. In: *Quantitative Analyses of Behavior, Vol. 7: Biological Determinants of Reinforcement and Memory*, A. Wagner (Ed.), 1986, in press.
- Stein, L. & Belluzzi, J.D. Cellular basis of positive reinforcement. In: *Anhedonia and Affect Deficit States*, D.C. Clarke (Ed.), SP Medical and Scientific Press, 1986, in press.
- Belluzzi, J.B. & Stein, L. Dopamine receptor regulation of single-unit operant conditioning in hippocampus. *Brain Research*, submitted.
- Trujillo, K.A., Belluzzi, J.D. & Stein, L. Opiate antagonists suppress self-stimulation behavior by actions in the central nervous system. *Behavioral Brain Research*, submitted.
- Trujillo, K.A., Belluzzi, J.D. & Stein, L. Opiate antagonists and self-stimulation: extinction-like response patterns suggest selective reward deficit. *Brain Research*, submitted.
- Trujillo, K.A., Belluzzi, J.D. & Stein, L. Naloxone suppresses both nose-poking and lever-pressing for self-stimulation. *Pharmacol. Biochem. Behav.*, submitted.
- Trujillo, K.A., Belluzzi, J.D. & Stein, L. Naloxone blockade of amphetamine-induced place preference conditioning. *Psychopharmacology*, submitted.

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